Docket No.: 61646 (70904)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Shunichi Shiozawa et al.

Application No.: 10/501,259 Confirmation No.: 7532

Filed: July 9, 2004 Art Unit: 1634

For: DISEASE SUSCEPTIBILITY GENE FOR RHEUMATOID ARTHRITIS, PROTEIN THEREOF, EVALUATION METHOD AND EVALUATION KIT FOR EVALUATING ONSET POSSIBILITY OF RHEUMATOID ARTHRITIS BY USING THOSE, AND REMEDY AND CURING MEDICINE FOR RHEUMATOID ARTHRITIS

Examiner: S.C. Pohnert

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.132

The undersigned, Shunichi Shiozawa, declares as follows:

1. I am a resident of Japan. I am an inventor or co-inventor of one or more of the claims of the above-referenced patent application ("the Application"). I have a Ph.D. degree from Kobe University Graduate School of Medicine and an M.D. degree from Kobe University Medical School.

2. I am familiar with the Application and with pending claims 4 and 11 thereof. I am also familiar with the Office Action issued from the U.S. Patent Office on December 5, 2008, in which the claims were rejected.

3. I am also familiar with certain experiments which were carried out in my laboratory.

4. In one experiment, PCR was performed as described below to obtain a partial sequence of Angiopoietin-1 from whole RNA of whole blood of RA patients and healthy subjects. The groups of subjects were selected from the general population in Japan.

By using an RNA extraction kit (Gentra Systems), total RNA was extracted from 300μl of whole blood from RA patients or healthy subjects. By reverse transcription reaction using an Oligo dT primer carried out in the usual method known in the art, a first strand cDNA was synthesized. With the cDNA used as a template, amplification of a region including a mutated site was carried out by a two-step RT-PCR using the following primers. The second-step PCR was carried out by using, as a template, 1μl of product obtained by the first-step PCR. A PCR product obtained by the second-step PCR was subjected to a sequence analysis and an analysis on long chain in the usual method known in the art, so as to determine whether or not the 3-base-insertion/deletion occurred at positions No. 805 to No. 807 in an Angiopoietin-1 gene.

First step

Sense primer: 5'-CCACCAACAACAGTGTCCTT-3'

Anti-sense primer: 5'-CAGCTTGATATACATCTGCACAG-3'

Second step

Sense primer: 5'-CAACCTTGTCAATCTTTGC -3'

Anti-sense primer: 5'-CAGCTTGATATACATCTGCACAG-3'

5. The results of the experiment performed in Paragraph 4 are shown in the Table below:

	RA (%)	Control (%)
No 3bp insertion	17 (11.7%)	17 (7.8%)
3bp insertion (Hetero)	59 (40.7%)*	133 (61.0%)
3bp insertion (Homo)	69 (47.6%)*	68 (31.2%)
N	145	218

* $p < 0.005$ (There is a significant difference.)
All of them are x^2 test results.

As shown in the Table above, the results of RT-PCR indicated that there was a statistically significant difference between healthy subjects and RA patients, in the proportion of subjects with mutation in homozygotes of the 3-base-insertion at positions No. 805 to No. 807 in Ang-I.

6. The data shown in the Table in Paragraph 5 include results from the subjects included in the data provided in my Declaration dated August 27, 2007 (submitted with an amendment dated September 11, 2007). However, the analytical methods used were different; the data provided in my Declaration dated August 27, 2007, were obtained by analyzing the PCR products with an older, less sensitive sequencing instrument, while the added data in the Table above were obtained using a higher performance sequencer. These differences in analysis may explain, at least in part, any differences in the data shown in Paragraph 5 as compared to the data provided in my Declaration dated August 27, 2007.

7. In my view, it is possible to evaluate the onset or onset possibility of rheumatoid arthritis in a human subject by detecting the homozygous presence or absence of a gene coding a protein comprising the amino acid sequence shown in SEQ. ID NO.:1 (as described in the present application) in the subject, because the presence

of the homozygous insertion mutation (the 3-base insertion at positions 805 to 807 in the nucleic acid sequence coding for Angiopoietin-1) is associated with rheumatoid arthritis, as described in the present application and in the experiment described herein.

8. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both (18 U.S.C. 1001), and that such willful false statements may jeopardize the validity of the above-identified Application or any patent issued thereon.

Date: April 15th 2009

Shunichi Shiozawa